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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

MICROBIOLOGICAL BACKGROUND*

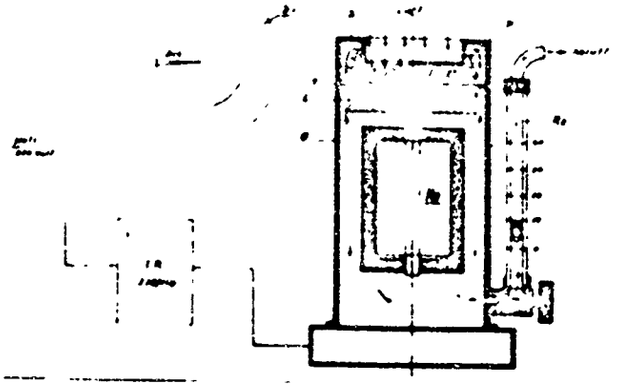
[Following is a translation of an article by W. Schiff, Hygiene Institute of Marburg University, from the German-language. Source unknown.]

The following action and experiments were carried out in the completion of the project of evaluating instruments for the sampling of air bacteria.

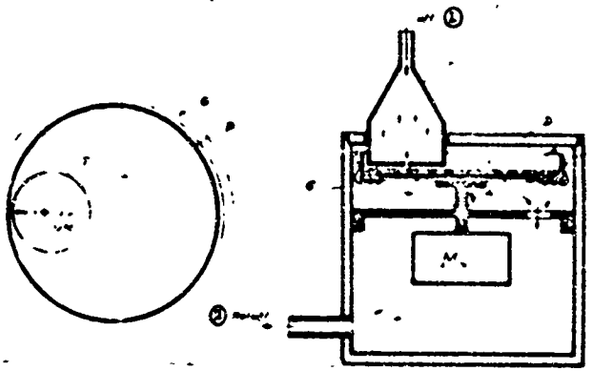
1. Training of assistants,
2. Establishment of a bacteriological research laboratory,
3. Supply, additions and new developments of equipment for the collection of air bacteria,
4. Quantitative comparative investigations with a total of eight different air collectors for bacteria in the laboratory,
5. Preliminary survey and investigations concerning the bacteria content in the higher layers of the air by means of an airplane.

As a result of late deliveries on the part of the industries and as a result of personnel difficulties (assistants with no previous bacteriological experience), we limited our investigations in the beginning mainly to the quantitative determination of the bacteria content in the closer vicinity (laboratory) by using basic and comparative experiments (see other portions of the text) which were carried out with the following eight different collecting instruments for air bacteria and which are shown schematically in Figure 1:

* 8. MWDDEA-A61-G68



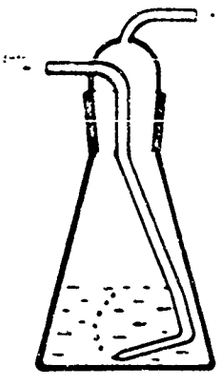
I. Krotov Instrument



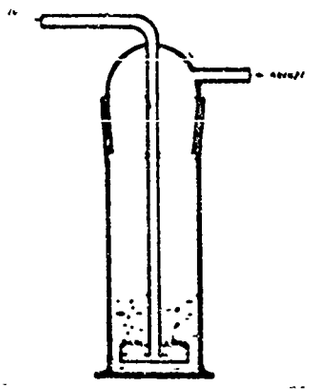
II. Slit-Sampler (Fort Detrick)

[Legend]: 1) exhaust air; 2) air.

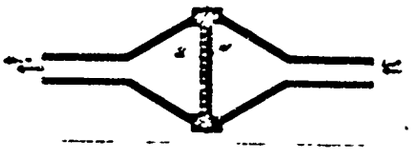
[Figure 1 continued on following page]



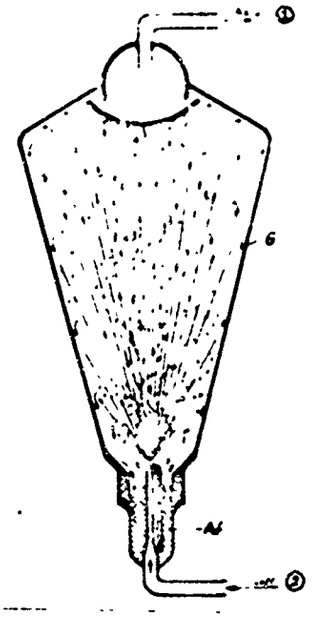
III. Capillary Impinger



IV. Fritted glass Impinger



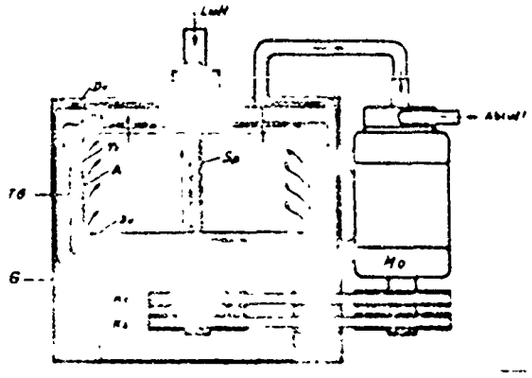
V. Membrane filter apparatus



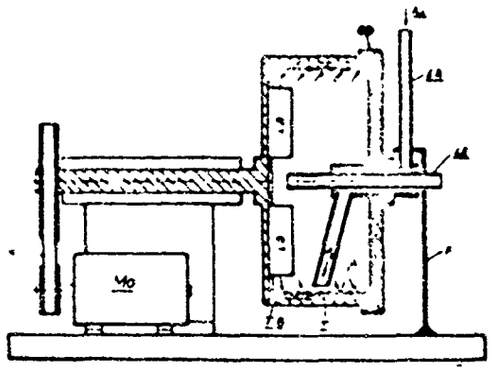
VI. Spray apparatus

[Legend]: 1) exhaust air; 2) air.

[Figure 1 continued on following page]

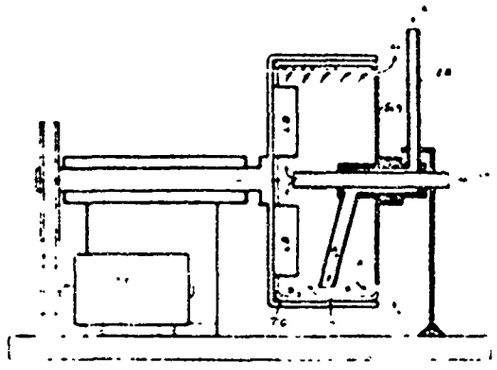


VII. Vertical centrifuge



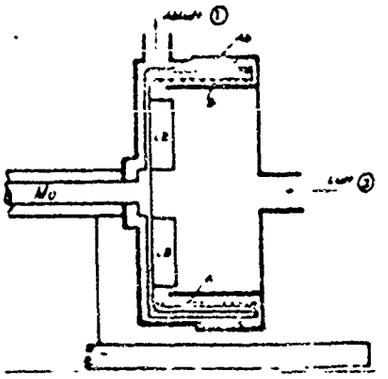
VIII. Horizontal centrifuge

a) agar drum pour apparatus

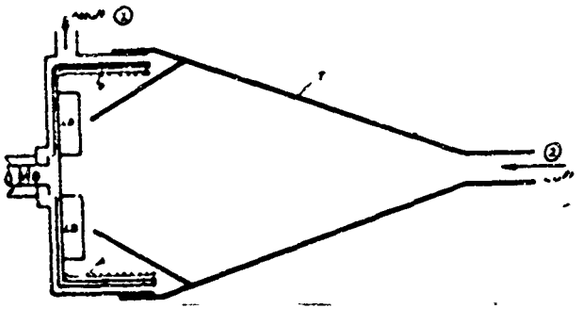


b) with immovable cover plate

[Figure 1 continued on following page]

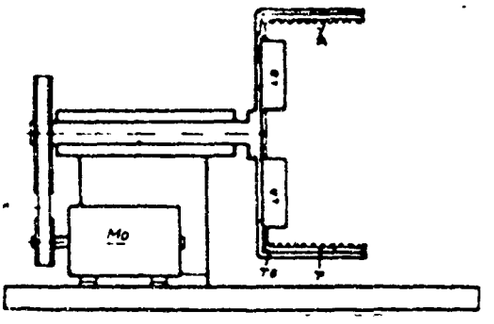


c) with immovable cover head



d) with immovable air intake funnel

[Legend]: 1) exhaust air; 2) air.



e) with free air access

Explanations:

A = Agar surface
A_1 = Agar introduction
A_b = cover head
A_f = collection liquid
A_l = exhaust air
D = lid, cover
Dü = mozzle
ER = filler pipe
F = fritted area
G = housing
K = wedge-belt
L = lamellae turn plate
LB = air foils
LE = air intake pipe
MF = membrane filter
Mo = Motor
P = petrodosj
Ro = rotameter
Sch = immobile cover disc
Schl = slit
SD = screw lid
Sp = spindle
St = support sieve
T = funnel
TG = drum housing
Tr = drum

Methods

The air flow which is required for the different procedures occurred in various ways: with the Krotov instrument (I) this occurred by means of a laminated turning disc which was built into the apparatus and with instruments II, V, VII (closed vertical centrifuge) by means of an aspirator fastened to the exhaust air line by which the quantity of air is regulated with a needle valve; for apparatus III, IV and VI we employed a water aspirator and while the resulting air suction was sufficient with the open vertical centrifuge containing the slot nozzle (VII) which rotates with it, we still used installed air leaves as ventilators for the apparatus VIIIa-e. The air volume measurements were carried out by means of a Wright respirometer having a measuring range of 100 liters or up to 10,000 liters of air or by means of a built-in rotameter having a measuring range of 20-40 liter per minute or 150 liters per hour) in the case of the Krotov instrument and the capillary impingers.

For the collection and cultivation of the air bacteria collected with the apparatus I, II, VII and VIII we generally used nutrient agar (Difco) with the addition of 5-7% defibrinated sheep blood; in this case the agar content was increased in the centrifuge drum from 1.5% to 2.5%. In the membrane filter instruments (membrane filter company, Gottingen) we used type MF gelatine (50 mm) as a filter which after use was placed into 30 ml of physiological sodium chloride solution for 30 minutes at 37°C; this liquid was then filtered through a membrane filter MF 100 (50 mm). Following this procedure these filters were placed on blood agar plates and were incubated. For both of the impinger procedures and the spray apparatus (apparatus III, IV and VI) a water solution containing 0.2% disodium hydrogen phosphate and 0.2% gelatin served as a collection liquid (150 or 50 ml). The bacteria found in the collection liquid were grown by means of pouring procedure with the use of nutrient agar and were then counted. In order to obtain reliable values with this procedure half of the collection fluid was generally used in parallel dilutions with a volume of 7.5 ml each. A count of the bacteria colonies was performed after an incubation of 48 hours on agar plates and drums or, in other words, after an incubation period of three days at 37°C. In case sterilization of the separate instruments, including accessories, was not possible in the autoclaves or in the hot air sterilizers, disinfection was carried out by means of a Bunsen burner flame.

Experimental Results

I. Determination of the Air Bacteria Content by Means of a Krotov Instrument

The first investigations were carried out with the Krotov instruments which were available to us at the end of 1964 and which had a maximal air flow of 30-35 liters per minute.

Since these instruments were very prone to breakdown, especially as far as their electrical parts were concerned, repairs were often necessary which lasted for several weeks and which often resulted in the installation of a new electrical motor. In this latter case a maximum air flow of 40 liters per minute could be obtained. Air samples smaller than 20 liters per minute were measured by means of an attached respirometer.

In the 45 comparative investigations listed in Table 1, the yield of air bacteria at an air flow of 5, 10, 20 or 40

liters per minute was examined comparatively over a period of several days. Since we only had two pieces of equipment available for the four different air samples volumes, we obtained a reference amount for each instrument when we adjusted it to different air sample volumes. Thus at an operation time of 10 minutes and with 50, 100, 200 or 400 liters total volume of the air samples we obtained on the average 280 or 190 per 330, or 270 or 165 bacteria per 33 mm of air. The deviation which was obtained here ranged between +250% and -65%. Because of these large scatter values which appeared also to a larger or smaller extent in the tests with other instruments, we had to resort to a larger number of single experiments in order to obtain reliable average values.

In the calculation of the relative percentage values it appeared that the best yield of bactera was obtained with the least airflow or five liters per minute. If this value is taken as 100% bacteria yield, then this amount is reduced respectively by about 68%, 54% or 43% when the airflow is raised to 10, 20, or 40 liters per minute. We did not examine any smaller air samples becaus this led to pronounced heating of the equipment as a result of a drastically reduced cooling effect due to reduced air flow. In addition the built-in rotameter failed with a small air volume. For this reason we generally used an optimum air flow of 20 liters per minute in the other subsequent examinations, which by necessity led to only half of the bacteria yield than if we had used an airflow of five liters per minute.

It seems that these relatively low values for the amounts of the air bacteria could be due to the very considerable heating of the instrument, especially as a result of the electrical resistances present in the housing. In order to elucidate this question we ran corresponding comparative tests in which we left one instrument (I) as before but substituted a variac (VT 220/110), which was connected from the outside to replace the resistances within the housing. In addition we measured the temperature of the instrument with a small thermometer which was fastened to the housing. As one can see in Table 2, the outside temperature of the housing of the test instrument rose only to 39°C after five immediately successive tests lasting 10 minutes each, while the temperature in the control instrument without the variac reached 62°C. In both cases however, we obtained an average count of 38 ot 36 bacteria per 200 liter of air. The very slight bacteria yield which was thus obtained in the preceding experiments could, therefore, not be explained bytoo high a heat production. However, in all subsequent experiments both Krotov instruments were used with an outside variac.

Table 1

45 Comparative Determinations of Air Bacteria Content by Means of Two Krotov Instruments Using Different Air Samples

Experimental room: small laboratory
Duration of experiments: 10 min. each

	ⓐ Krotov-Gerät					
	I	II	I	II	II	I
	ⓑ Luftdurchfluß pro min.					
	5 l	10 l	10 l	20 l	20 l	40 l
	ⓒ Keimzahlen pro 10 min. Laufzeit					
	19	14	24	30	55	46
	5	10	35	59	36	36
	11	19	42	55	34	82
	14	18	35	71	15	43
	22	54	35	28	29	48
	33	37	28	44	34	40
	12	21	28	40	33	70
	19	34	14	40	48	44
	14	15	28	38	22	41
	7	8	17	37	80	145
	13	11	53	122	54	117
	14	13	21	22	61	90
	9	14	9	28	32	60
	18	14	83	85	54	24
	5	9	46	105	41	82
ⓓ Mittelwerte	14	19	33	54	42	66
ⓔ Mittelwerte /m^3 Luft	280	190	330	270	210	165
ⓕ in rel. %	100	68	68	56	56	44

[Legend: a) Krotov Instrument; b) air flow per min; c) Bacteria count per 10 min. of operation; d) average values; e) average values per m^3 air; f) in rel. %.

Table 2

Comparison of Air Bacteria Yield With the Krotov Instrument with Different Degrees of Heating (with or without Outside Variac)

Experimental room: small laboratory
Duration of experiment: 10 min. each
Air Flow: 20 l/min.

ⓐ Vers.-Nr.	ⓑ Gehäuse-Temperatur	ⓒ K I (orig) (ohne Trafo) Keimzahlen /200 l	Gehäuse-Temperatur	K II (mit Trafo) Keimzahlen /200 l
1	20° C	28	20° C	22
2	48° C	64	31° C	54
3	57° C	53	36° C	57
4	60° C	18	36° C	19
5	62° C	26	39° C	27
ⓓ Mittelwerte /200 l Luft		38		36

[Legend]: a) trial No.; b) housing temperature; c) K I (orig) without variac -- No. of bacteria per 200 l; d) average values per 200 l air.

We then suspected that a large portion of the air bacteria may not even be collected by the agar plate, thus we checked the amount of bacteria in the exhaust air with and without the inserted agar plate (at an airflow of ten liters per minute) by means of a membrane filter apparatus (soluble gelatine filter). The results listed in Table 3 show that this exhaust air is quite free of bacteria, yet we

have to consider that according to the experiences of other authors using this filter process, the destruction of the vegetative bacteria can occur by dessication or when they hit the agar plate. Strangely enough, however, the outgoing air was just as free of bacteria when no agar plate was introduced. We obtained similar results when we sampled the outgoing air by means of a slit sampler. The cause for the disappearance of the bacteria within the apparatus still remains unexplained. Thus testing the exhaust air gives no indication as to the efficiency of the Krotov apparatus.

Table 3

Determination of air bacteria content in the exhaust air of the Krotov instrument by means of a membrane filter (MF)
6 comparative experiments

Experimental room: large laboratory
air flow: 10 l/min
duration of experiment: 10 min. each

ⓐ Krotov - Gerät		
ⓑ mit Agarplatte		ⓔ ohne Agarplatte
ⓒ Zuluft (Agarplatte)	ⓓ Abluft (MF)	Abluft (MF)
ⓕ Keimzahlen		
11	1	0
12	0	0
21	0	0
86	0	1
20	0	2
32	0	0
ⓖ Mittelwerte/m^3 Luft		
300	2	5

[Legend]: a) Krotov instrument; b) with agar plate; c) intake air (agar plate); d) exhaust air; e) without agar plate; f) number of bacteria; g) average values per m^3 air.

Considering the large variation of the values for the air bacteria as listed in Table 1 and considering the necessity of carrying out air bacteria counts at different times of the day, closer knowledge of the daily variations of these bacteria was desirable. We, therefore, used these two Krotov instruments during eight different experimental days at two hour intervals to establish the air bacteria content of our laboratory area. During the tests, this area, which had an approximate volume of 95 m^3 and was located on a major traffic artery, contained on the average one to four persons and the windows remained closed at all times. The average values which we determined per m^3 air are presented graphically in Figure 2. From these results we can see that even though considerable deviations and, under the given conditions, short term deviations occurred, the bacteria count in the a.m. period was definitely higher than in the p.m. period and the evening. Thus, the bacteria count decreased from an average of 415 around 8:00 a.m. to an average of 68 about 10:00 p.m. During this time interval the relative bacteria count thus was reduced by about 84%. In similar preliminary orientation investigations with the same apparatus in a smaller laboratory which contained no people (volume 34 m^3) as well as in the outside open air with no traffic we obtained decreased values in the bacteria count during the same time interval as shown by a decrease of 125 - 65 or 200 - 40 bacteria per m^3 air. These small bacteria yields left us with the impression that the Krotov apparatus is little suited for the satisfactory determination of the amounts of bacteria in the air.

Since the collection instruments used in the determination of the bacteria content were constantly in use with only short term interruptions, we also checked the possibility of whether the duration of the time the instruments were in use had any effect on the different yields of bacteria. For this purpose we carried out successive determinations of the air bacteria content lasting five, ten and twenty minutes by using two Krotov instruments, both of which had exactly the same settings, but one instrument was operated in the indicated sequence and the other one in the reverse sequence. The single and average values which were obtained in 10 such experiments are given in Table 4. Considering the operation times which differed by a factor of two, we should, theoretically, have obtained bacteria counts in the proportions of 1:2:4 (or in the reverse).

However, as shown in Table 5 the average values for the bacteria counts with decreasing experimental duration corresponded only to the proportion of 0.86:2.21:4 in comparison with the theoretical proportions, while with increasing operation time, proportions of 1:1.7:2.1 were obtained.

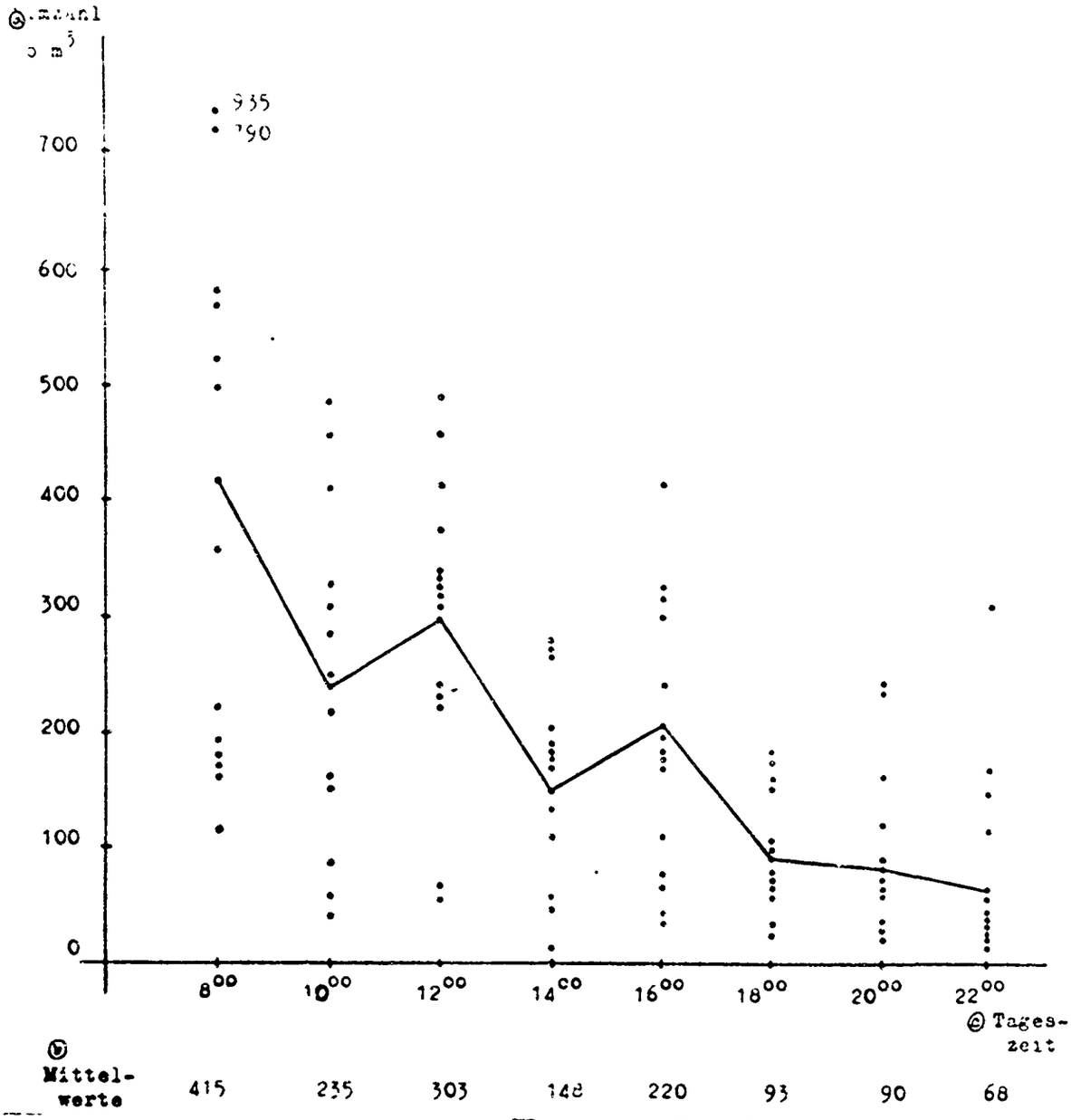


Fig. 2. Daily fluctuation of the air bacteria content (Single and mean values of 8 experimental days, each obtained with 2 Krotov instruments)

Experimental area: large laboratory
Air flow: 20 l/min.
Experimental Duration: 10 min. each

[Legend]: a) No. of bacteria per m^3; b) mean values; c) time of day (based on 24 hrs.

Table 4

Air Bacterial Yield with the Krotov Instrument in Successive, Decreasing or Increasing Sequence

Experimental Area: small laboratory
Air flow: uniformly 20 l/min

	Krotov I	Krotov II	Krotov I	Krotov II	Krotov I	Krotov II
	ⓑ K e i m z a h l e n					
ⓐ Versuchs-Nr.	ⓒ Versuchsdauer 5 min.		10 min.		20 min.	
1	61	64	99	126	151	227
2	51	60	103	103	81	54
3	45	48	72	66	83	100
4	57	45	77	81	91	97
5	44	32	61	78	102	89
ⓓ Mittelwerte	52	50	92	91	102	113
	51		86,5		107,5	
	Versuchsdauer 20 min.		10 min.		5 min.	
6	430	324	247	222	80	80
7	215	189	125	121	70	37
8	100	96	29	45	17	14
9	91	55	31	30	11	22
10	47	52	15	24	8	9
Mittelwerte	177	143	89	88	37	32
	160		88,5		34,5	

[Legend]: a) ExperimentNo.; b) No. of bacteria; c) Experimental duration; d) mean values.

These results show that by operating the Krotov instruments for a longer time the yield is decreased. Since according to our results listed in Table 3 this decrease cannot be explained by an increasing heating effect, and since the demonstrated short term deviations (see Fig. 2) are not sufficient for an explanation, other reasons must be involved. For these reasons these experiences call for a reconsideration of the efficiency of the Krotov instrument.

Obviously the different ages of the solid nutrient media cannot be responsible for such different bacteria yields, for coinciding counts were obtained in similar comparative investigations in which freshly poured and over twenty-four hour old blood agar plates were used.

Table 5

Relationship of the Theoretical to the Actual Bacteria Yield of the Earlier Mean Values

	ⓓ Versuchsdauer		
	5 min.	10 min.	20 min.
ⓐ Theoretisch	1	2	4
ⓑ tatsächlich, bei zunehmender Versuchsdauer	1	1,70	2,10
" ⓒ , bei abnehmender Versuchsdauer	0,86	2,21	4

[Legend]: a) Theoretical; b) Actual with increasing experimental duration; c) actual with decreasing experimental duration; d) Experimental duration.

During the investigation of five commercial nutrient medium bases for the production of the blood agar it was shown that the use of tryptose agar (Difco) yielded twice as many bacteria as extract agar (Difco), standard--I-nutrient agar (Merck) and the common nutrient agar. Nutrient agar (Difco) was only slightly worse than tryptose agar.

II. Determinations of the Air Bacteria Content by Means of Slit Samplers (Fort Detrick)

In the middle of March 1965 we had two instruments

available which were built according to the specifications of the American slit samplers (Fort Detrick) (see Fig. 1, and II). The clock work running for 60 minutes per turn (for the transport of the bacteria collecting agar plate) which was present in the original instrument, was replaced by one which had a rotating time of 15 minutes. For the measurement of the air flow we installed into the exhaust line a Wright respirometer, which was connected to the suction pipe by means of an aspirator. In the American literature an optimal airflow of 28 liters per minute at a slit width of about 0.2 mm is reported.

The preliminary determinations of the air bacteria content performed with these two instruments took place with an air flow of 20 liters per minute and a slit width of 0.2 mm. As a control similar, equally large air samples were tested with the two Krotov instruments. The average values for the bacterial content in the air which were obtained in 19 of such comparative double experiments are shown in Table 6. Under the given conditions the slit samplers had about one third less of a bacteria collecting capacity than the Krotov instruments, i.e. the slit samplers yielded 144 bacteria per m^3 air (i.e. 28.8 per 200 liters) as compared to the 214 obtained by the Krotov instruments.

Since these two instruments essentially only differ functionally in that the agar plates have different rotational speeds and in that there is a different air supply with or without a funnel, we suspected that this may be the cause for the different bacteria yields. For this reason we substituted the original clockwork in the housing of the slit sampler by a Kleinst electromotor, in order to equate it functionally to the Krotov instrument. We compared the efficiency of the original slit sampler with that of the modified one by using an equal air flow of 20 liters per minute at 10 minutes operation time. In a total of 41 such simultaneous comparative experiments we did, however, obtain coinciding yields of bacteria, which, on the average, amounted to 102 to 106 bacteria per m^3 of air. These results let us conclude that the differing rotational speeds of the agar plates essentially do not cause for the different yields in bacteria in the slit sampler and the Krotov instrument.

For this reason we subsequently examined the extent to which the efficiency of the slit sampler was dependent on the air supply, i.e. the adjustment of the apparatus as far as slit to agar distance, slit width and air flow are concerned. In order to have a reference quantity, we also used a second instrument for comparison purposes which had a slit to agar distance of 3 mm, a slit width of 0.2 mm and an air

Table 6

Comparative Determinations of the Air Bacteria Content with the use of Two Slit-samplers (Fort Detrick) and Two Krotov Instruments

Experimental Area: small laboratory
Experimental Duration: 10 min.
Air Flow: [0 l/min.

ⓐ Versuchs-tag	Slit-Sampler +) I	Slit-Sampler +) II	ⓑ Krotov-Gerät I	ⓑ Krotov-Gerät II
	ⓒ K e i m z a h l e n			
ⓓ 10. 5.	47	48	67	56
	60	52	56	66
	35	32	72	62
	38	60	43	58
ⓔ 19. 5.	43	49	35	61
	24	46	26	38
	38	44	62	38
	38	36	53	51
	37	28	41	33
ⓕ 26. 7.	23	26	32	47
	21	20	31	32
	28	14	52	56
	15	14	50	31
	20	14	29	38
ⓖ 27. 7.	10	19	39	44
	9	6	20	19
	11	9	17	16
	20	26	57	39
	19	17	20	35
ⓗ Mittelwerte /200 l Luft	28,2	29,4	42,2	43,2
	28,8		42,7	

+ air flow 20 l/min.
slit width 0.2 mm
slit/agar distance 3 mm

[Legend]: a) experimental day; b) Krotov Instrument; c) No. of bacteria; d) May 10; e) May 19; f) July 26; g) July 27; h) mean values per 200 l of air.

flow of 10 liters per minute. The values for the bacteria count and the comparison values obtained in a total of 81 such comparative investigations showed a wide range similar to that obtained with the Krotov instrument (see Table 1): the minimal and maximal single value amounted to 38 or 1,780 bacteria per m^3 of air (each at an airflow of 40 liters per minute). In Table 7 are listed the average values which were obtained in the different settings and which are reported as a percentage of the corresponding comparison values (100%). Thus it is obvious that the best results are obtained with a slit/agar distance of 3 to 6 mm, a slit width of 0.4 mm and an airflow of 5 liters per minute; these setting gave a relative bacteria yield of 180 to 175%. For our subsequent investigations we, therefore, adjusted our instrument always to a slit/agar distance of 3 mm, a slit width of 0.4 mm and an airflow of 5 liters per minute; this was in contrast to the recommendations by the American authors. Since this instrument is driven by a clock work, there is no danger of any excessive heat production.

The results of our investigations listed in Table 6 and 7 let us conclude that among these last conditions the slit sampler (Fort Detrick) is comparable to the Krotov instrument as far as efficiency goes, but only when a non-optimal air flow of 20 liters per minute occurs in this latter instrument. This has also been supported in subsequent investigations in order to find out what effect the air supply funnel has on bacteria yield. For this purpose the air supply funnel was removed from the slit sampler and one of the Krotov instruments (originally the one without a funnel) was supplied with an equally large air supply funnel. Table 8 lists the average bacteria count obtained per cubic meter of air which was obtained in a total of 25 similar trials with 4 operational bacteria collectors being used at each run. These results show that with an optimal air flow of 5 liters per minute the efficiency of the original slit sampler with the funnel and that of the original Krotov instrument without the funnel were comparable; in both cases an average of 172 or 164 bacteria per m^3 air was obtained. On the contrary, without a funnel the slit sampler is considerably superior to

Table 7

81 Comparative Determinations of Air Bacteria Content by means of a slit-sampler (for Detrick) by Employing various slit/agar-distances, slit widths, and air sample volumes

(Mean values of Bacteria Number in % of each mean value of the control (= 100%)

Experimental area: large laboratory
operation time: uniformly 10 min.

Uniform Setting of the Control Instrument:

Slit/agar distance 3 mm
slit width 0.2 mm
air flow 10 l/min.
operation time 10 min.

ⓐ Schlitz-breite mm	ⓑ Luftmenge in l/min. 2,5	5	10	20	40
	ⓒ Schlitz/Agar-Abstand 3 mm				
	*)	***)			
0,1	154 %	87 %	130 %	.	.
0,2	147 %	135 %	126 %	57 %	59 %
0,4	113 %	180 %	72 %	55 %	23 %
0,6	150 %	152 %	96 %	67 %	35 %
0,8	85 %	169 %	82 %	34 %	57 %
	ⓒ Schlitz/Agar-Abstand 6 mm				
	*)	**)			
0,1	140 %	98 %	72 %	.	.
0,2	149 %	131 %	70 %	121 %	34 %
0,4	140 %	175 %	34 %	46 %	48 %
0,6	161 %	125 %	58 %	65 %	63 %
0,8	128 %	107 %	82 %	86 %	29 %

* Average values of 2 comparative experiments
** Average values of 3 comparative experiments
*** Average values of 4 comparative experiments

[Legend]: a) slit width; b) air volume in l/min; c) slit/agar distance.

Table 8

Comparative Determinations of the Air Bacteria Content with 2 slit-samplers and two Krotov instruments with and without air supply funnel

(Mean values of 25 comparative experiments)

Experimental area: large laboratory
Experimental duration: uniformly 10 min.

ⓐ Luftdurchfluß pro min.	Slit-Sampler 5 l		ⓑ Krotov-Gerät 20 l		ⓒ 5 l (theoretisch)+)	
ⓓ Trichter	ⓔ mit	ⓕ ohne	mit	ohne	mit	ohne
ⓖ Keimzahl-mittelwerte m^3 Luft	172	207	136	164	(255)	(304)

* compare Table 1

[Legend]: a) air flow per min; b) Krotov-instrument; c) theoretical; d) funnel; e) with; f) without; g) mean values of the number of bacteria per m^3 air.

the Krotov apparatus as shown by a count of 207 bacteria per m^3 of air in the slit sampler as compared to 164 bacteria per m^3 of air in the Krotov instrument. With the installation of a funnel (opening 10 mm) the bacteria collecting capability decreased 17% in both instruments. This, however, is not unimportant since in the determination of air bacteria by means of an airplane a funnel can hardly be avoided. This conclusion, which is derived from comparative experiments, is, however, only valid for a Krotov instrument with an air flow of 20 liter per minute. If 46% higher bacteria yield is considered at an optimum air flow of 5 liters per minute, (compare Table 1), which we did not carry out because of the danger of overheating the electromotor, we

find a definite superiority of the Russian compared with the American instrument including also the case where the air supply occurs by means of a funnel.

With the slit sampler we had the same experience as before with the Krotov instrument (see Table 4) in that the bacteria yield of the instrument is impaired by extended operation times. When the apparatus was successively operated for 2, 5, 5 and 10 minutes, as well as in the reverse sequence, an approximation of the theoretical bacteria yield per portion of 1:2:4 was only obtained in the latter case with values of 0.8:1.7:4, whereas in the case of increasing operation times a larger deviating proportion of 1:1.2:2.7 resulted.

In spite of the proven lack of bacteria in the exhaust air of the Krotov instrument (see Table 3) there was no doubt that with such instruments only small portions of the air bacteria are collected. Since the determination of the bacteria content in the exhaust air is an important criterion for the efficiency of an instrument, we also carried out such investigations with the slit sampler. For these tests we employed the capillary impinger apparatus (see Figure 1 III), since a better bacteria yield can be obtained with it than with the slit sampler; the use of the capillary impinger is especially indicated with the slit sampler because it can be adjusted without difficulty for a smaller air flow which is necessary for the impinger procedure. With an air sample volume of 5 liters per minute and an experimental duration of 1 hour, the exhaust air was divided equally (each at 1.25 liters per minute) on the four impingers which were mounted in parallel. The results of five such experiments are given in Table 9. Whereas the slit samplers with 4 agar plates and an operation time of 15 minutes each, yielded, on the average, 192 bacteria per m^3 of air, the exhaust air still showed 276 bacteria per m^3. According to these results, the slit sampler only took in about 41% of all the bacteria which were collected. Further exhaust air tests were performed with three slit sampler connected in parallel (1 slit sampler and 2 Krotov instruments, exhaust air flow 15 liters per minute each), in which case the introduced slit sampler was adjusted to an air sample volume of 45 liters per minute. With these instruments we only showed about 10% of the primary yield in the exhaust air. Thus one can see that the impinger method is definitely superior to the method employing agar plates.

Table 9

Determination of the air bacteria content in the exhaust air of the slit-sampler by means of four capillary impingers connected in parallel

Experimental area: large laboratory
Air flow through the slit sampler: 5 l/min.
Experimental duration: 1 hr.

	Keimzahlen							
	Zuluft (Slit-Sampler)		Abluft					
Vers.-Nr.			Kapillar-Impinger				Summe von I_1-I_4	
	pro Std.	pro m^3	I_1	I_2	I_3	I_4	pro Std.	pro m^3
1	16	53	45	9	27	18	99	327
2	48	160	18	15	21	24	78	260
3	111	370	21	12	12	12	57	190
4	43	141	39	15	15	21	90	297
5	71	234	27	12	6	18	63	308
Mittel-werte		192						276
in %		41						59

[Legend]: a) Number of bacteria; b) Air supply (slit-sampler); c) exhaust air; d) Experiment #; e) per hr; f) Capillary-impinger; g) sum of; h) mean values.

III. Determinations Of Air Bacteria Content By Means of Capillary Impingers

Thus, it seemed advisable to further test the impinger process for its efficiency. For this reason we chose original Bronn capillary impingers (see Fig. 1, III), which,

according to Windisch (Zb., Bakt. I Orig. 1966, in print) are supposed to make an air bacteria yield of 98% possible.

First we examined the extent to which the bacteria yield was dependent upon the quantity of the air bubbling through the instrument containing 150 ml of a collecting liquid. We examined the bacteria yield at an air flow of 25, 50, and 100 liters per hour with a total of six comparative duplicate experiments; since we had only two instruments available which contained a built-in rotameter, the middle setting always served as our reference amount (bacteria yield always set at 70%). The results presented in Table 10 show as expected, that similar to the Krotov instrument and the slit sampler, a larger bacteria yield can be obtained with less air flow than with larger air sample volumes. Thus the yield obtained with 25, 50 and 100 liters per hour were respectively 967,680/620 and 497 bacterial per cubic meter which in a percentage proportion would be 100 : 70 : 56.

On the contrary the volume of the collecting liquid influences the effectivenessof the capillary impinger far less. Thus we obtained on the average 660 or 546 bacteria per m^3 of air in five parallel experiments using liquid volumes of 150 and 60 ml. Contrary to the volume of 60 ml., recommended Windisch, we systematically used 150 ml. of collecting fluid in the instrument.

We compared the efficiency of the capillary impinger with that of the slit sampler and the Krotov apparatus by using an air flow of 50 liters per hour or 0.85 liters per minute. The average values obtained with a total of five similar air bacteria determinations are given in Table 11. According to these data a bacteria yield which was 4 to 5 times greater than that obtained with the American or Russian instruments was obtained with this instrument.

In order to better determine the efficiency of the Bronn capillary impinger we checked the exhaust air of such an instrument having an air flow of 50 liters per hour, by means of five other similar instruments which were connect in series. The bacteria counts obtained with a total of eight similar experiments are presented in Table 12. These data show that only a certain portion of the air bacteria are taken up by all of the 6 capillary impingers and that with this apparatus still only about 40% of the bacteria yield is obtained in comparison with the other instrument. Considering the tendency toward a decreased count from the five exhaust air controls we can conclude that under the given conditions only about 5-10% of the aerophilic air

Table 10

Determination of Air Bacteria with Two Capillary Impingers with Various Air Flows

(Mean values of six comparative experiments)

Experimental area: large laboratory
Experimental duration: uniformly 1 hr.
Air Flow: 25, 50 or 100 l/h.

	ⓑ Kapillar-Impinger I	II	II	I
ⓐ Luftdurchfluß pro Std.	25 l	50 l	50 l	100 l
ⓒ Keimzahlen/m^3 Luft im Mittel	967	680	620	497
ⓓ in rel. %	100	70	70	56

[Legend]: a) air flow per hour; b) capillary impingers; c) Number of bacteria per m^3 of air in rel. %; d) in rel. %.

bacteria content is obtained with one single capillary impinger. This would mean that the tested air contained approximately 4,000 bacteria per m^3. These results seem in drastic contrast to the estimate by Windisch and thus call for an explanation.

IV. Determinations Of the Air Bacteria Content By Means Of the Newly Developed Impinger

We have developed an impinger instrument which functions on the same principle as the capillary impinger but in which a commercial fritted glass plate (emersion suction apparatus G1, Schott and Gen Company, Mainz) is welded to the air intake pipe so that a large number of finely divided air bubbles pass through the collecting liquid. In comparative parallel experiments with the Bronn capillary impingers under the same conditions we found (see Table 13), that a 30% higher bacteria yield can be obtained with our instrument.

Since we only had one single fritted glass impinger of this type available, exhaust air tests were carried out

Table 11

Comparative Determinations of Air Bacteria Content With Capillary Impinger, Slit Sampler and a Krotov Instrument

(Mean values of 5 comparative experiments)

Experimental area: large laboratory
Experimental duration: uniformly 1 hr.

	ⓐ Kap.-Impinger	ⓑ Slit-Sampler (4 Läufe à 15 min.)	Krotov-Gerät (4 Läufe à 15 min.)
ⓒ Luftdurchfluß	0,84 l/min. (50 l/h)	5 l/min.	20 l/min.
	ⓓ K e i m z a h l e n		
ⓔ pro 1 Std.	58	80	232
ⓕ pro m^3 Luft	1160	267	193
ⓖ relative Keimausbeute in %	100	23	17

[Legend]: a) cap. impinger; b) 4 runs; c) air flow; d) Number of bacteria; e) per hr.; f) per m^3 air; g) relative bacteria yield in %.

with 6 capillary impingers which were connected in series. The results presented in Table 14 verify the experience gained with a capillary impinger (see Table 12) that in spite of the relatively high efficiency of such instruments only a small portion of the air bacteria can be demonstrated. In the present case still over 50% of the bacteria obtained with a fritted glass impinger was collected from the exhaust air of the latter, i.e. the sixth control impinger.

Since the exhaust air control values did not show any strong tendency for decrease, these tests yielded somewhat better data about the efficiency of the fritted glass impinger. The bacteria collecting ability of this apparatus should with a high degree of certainty not be more than 10% of the actual content of the air.

Table 12

Determinations of the Air Bacteria Content in the exhaust air of capillary impingers by means of 5 capillary impingers connected in series

Experimental area: large laboratory
air flow: 50 l/h (=0.84 l/min.)
experimental duration: each 1 hr.

ⓐ Vers.-Nr.	ⓑ Zuluft	ⓒ Abluft					
	Kapillar-Impinger						ⓓ Summe von I_2 - I_6
	I_1	I_2	I_3	I_4	I_5	I_6	
	ⓔ Keimzahlen						
	ⓕ pro Std.	ⓖ pro m^3 Luft	ⓗ pro Std.				pro Std. / pro m^3 Luft
1	42	840	33	15	33	.	.	81	1620
2	45	900	21	21	15	.	.	57	1140
3	27	540	24	24	27	.	.	75	1500
4	24	480	12	15	21	9	9	66	1320
5	78	1560	129	72	78	30	30	339	6780
6	36	720	24	42	60	18	21	165	3300
7	21	420	15	15	12	12	12	66	1320
8	21	420	15	12	15	6	9	57	1140
ⓘ Mittelwerte	37	740	34	27	32	15	16	113	2260
in rel. %		24,5							75,5

[Legend]: a) Experimental No; b) Supply air; c) exhaust air; d) sum of; e) No. of bacteria; f) per hr; g) per m^3 air; h) per hr; i) mean values.

Table 13

Comparative Determinations of Air Bacteria Content by means of a capillary impinger and a fritted glass impinger

Experimental area: large laboratory
Experimental duration: 1 hr
Collection liquid: each 150 ml
Air flow: 25 l/h

ⓐ Vers.-Nr.	ⓑ Kapillar-Impinger		ⓒ Fritte-Impinger	
	ⓓ Keimzahlen			
	ⓔ pro Std.	ⓕ pro m^3 Luft	pro Std.	pro m^3 Luft
1	39	1560	57	2280
2	57	2280	51	2040
3	24	960	27	1080
4	36	1440	48	1920
5	24	960	27	1080
6	36	1440	48	1920
7	48	1920	54	2160
8	30	1200	66	2640
Mittel-werte ⓖ		1470		1890
in %		100		129

[Legend]: a) experimental no.; b) capillary impinger; c) fritted glass impinger; d) No. of bacteria; e) hr; f) per air; g) mean values.

V. and VI. Determinations of the Air Bacteria -- Content With Membrane Filter and Spray Instruments

Since membrane filter instruments or air-water-spray instruments are used for the examination of the bacteria

Table 14

Determination of the Air Bacteria Content in the Exhaust Air of the Fritted Glass Impinger by Means of Six Capillary Impingers Connected in Series

Experimental area: large laboratory
air flow volume: 25 l/h
experimental duration: one hr. each

a) Vers.-Nr.	b) Zuluft	c) Abluft							
	d) Fritte-Impinger	e) Kapillar-Impinger						f) Summe von I_1 – I_6	
		I_1	I_2	I_3	I_4	I_5	I_6		
		i) Keimzahlen							
	g) pro Std.	h) pro m^3 Luft	pro Std.					pro Std.	pro m^3 Luft
1	27	1080	16	11	12	10	9	15	84	3360
2	15	600	15	14	12	9	9	9	66	2640
3	30	1200	21	16	16	15	10	21	111	4440
4	33	1320	27	21	24	15	19	19	129	5160
5	18	720	19	6	12	15	10	15	84	3360
j) Mittelwerte	25	984	20	13	16	14	11	16	95	3800
in %		20,6								79,4

[Legend]: a) experiment no.; b) intake air; c) exhaust air; d) fritted glass impinger; e) capillary impinger; f) sum of; g) per hr; h) per m^3 air; i) No of bacteria; j) mean values.

content of the air, and since these procedures have been described in the literature as being quite suited for these purposes, we also have included these two methods in our examinations.

With a total of six simultaneous comparative experiments involving a membrane filter instrument, a slit sampler and a Krotov instrument, we determined the average bacteria counts listed in Table 15. According to these results the relative bacteria yield of the rather troublesome and awkward membrane filter process was only 38% that of the slit sampler. With the Krotov instrument a relative bacteria yield of 67% was obtained, but one should once again consider the relatively large air flow volume of 20 liters per minute whereby the bacteria collecting ability is strongly diminished (compare Table 1).

Table 15

Simultaneous comparison of membrane filter, slit sampler and Krotov instrument

(Mean values of 6 experiments)

Experimental area: large laboratory
experimental duration: uniformly 10 min.

	ⓐ Membran-Filter	ⓑ Slit-Sampler	ⓒ Krotov-Gerät (ohne Trichter)
ⓓ Luftdurchfluß	10 l/min.	5 l/min.	20 l/min.
	ⓔ K e i m z a h l e n		
ⓕ pro 10 min. Gesamtlaufzeit	9	12	32
ⓖ pro m^3 Luft	90	240	160
ⓗ relative Keimausbeute in %	38	100	67

[Legend]: a) Membrane filter; b) slit-sampler; c) Krotov instrument (without funnel); d) air flow; e) No. of bacteria; f) per 10 min. of operation time; g) per m^3 air; h) relative bacteria yield in %.

Four capillary impingers connected in parallel with an air supply of 1.25 liters per minute each, were installed at the exhaust line for the determination of the efficiency

of the membrane filter apparatus having an air supply of 5 liters per minute. Table 16 lists the average bacteria count of a total of four such experiments lasting 30 minutes each.

With a relative bacteria yield of 32% the exhaust air contained at least twice as much bacteria as was collected by the installed membrane filter. In reality the bacteria yield with this instrument is undoubtedly very much lower since we have shown with the impingers connected in series (see Tables 12 and 14) that with one single impinger instrument with an air flow of 1.25 liters per minute, more or less only about five to 10% of the air bacteria was collected. We can thus conclude that in the present case the air can be estimated to contain 3,000 to 6,000 aerophilic bacteria per m^3 of air. Similarly, unsatisfactory results were obtained with a spray apparatus of our own construction (see Fig. 1, VI).

Table 16

Determinations of bacteria in the intake air and exhaust air by means of a membrane filter or four capillary impingers connected in series

(Average values of 4 experiments)

Experimental area: large laboratory
Experimental duration: each 30 min.

	ⓑ Membran-Filter	© Abluftkontrollen mittels parallelgeschalteten 4 Kapiller-Impingern
ⓐ Luftdurchfluß	5 l/min.	4 x 1,25 l/min.
	ⓓ Keimzahlen	
ⓔ Mittelwerte pro 30 min.	21	45
ⓕ Mittelwerte pro m^3 Luft	140	300
ⓖ Relative Keim-ausbeute in %	32	68

[Legend on following page]

[Legend]: a) air flow; b) membrane filter; c) exhaust air controls by means of 4 capillary impingers connected in series; d) No. of bacteria; e) mean values per 30 min; f) mean values per m^3 air; g) relative bacteria yield in %.

The development of this instrument originated from the concept that in the permanent atomization of a certain small liquid volume by means of the air to be tested, a greater loss in bacteria will occur in the collecting fluid For this purpose we installed a liquid atomizer at the base of a pear-shaped glass dome which widened toward the top. This way the collection fluid is sprayed into the glass dome in the form of very fine droplets but will return along the glass walls back to the original container. The exhaust or exit pipe was installed in a "dead space" at the upper end of the dome. With optimal atomizing functions this instrument could be operated at approximately 450 liters of air per hour. When the bacteria count is calculated we have of course to take into consideration the loss in liquid volume which amounts to approximately 1/3 of the original volume per hour.

With this instrument we have undertaken five comparative experiments with the capillary impinger and the slit sampler. The data obtained for the average bacteria counts are given in Table 17. According to these data this spray apparatus possesses only an efficiency of about 34% in comparison with a single capillary impinger. At the present time a modified instrument is being built in which the exit pipe comes off the "dead space" at the lower end of the glass dome and thus is opposed to the direction of the atomization.

VII. Determinations Of Air Bacteria Content With A Vertical Centrifuge Developed In Our Laboratory

A few authors have described instruments for the collection of air bacteria in which the bacteria are collected by means of the centrifugal force produced by the rapidly rotating drums on whose inner side an agar or liquid layer is present (Wells, 1933; Safir, 1945). On the other hand, Anderson (1962) has described a collecting instrument in which the air is forced with a jet onto the outside of a slowly rotating and rising agar drum.

After incubation the bacteria thus collected from colonies which are arranged on the drum in a spiral manner. This

Table 17

Simultaneous Comparison of Spray Apparatus, Capillary Impinger and Slit Sampler

(Mean values of 5 experiments)

Experimental area: large laboratory
experimental duration: uniformly 1 hr.

	ⓑ Spray [illegible]	ⓒ Cap.-Impinger	Slit-[illegible]
ⓐ Luftdurchfluß	490 l/h	50 l/h	ⓔ 5 l/1 m (in front of Platten pro Versuch)
	ⓔ Keimzahlen		
ⓕ pro 1 Std. Gesamtluftmenge	80	20	75
ⓖ pro m^3 Luft	170	520	230
ⓗ relative Keimausbeute in %	34	100	40

[Legend]: a) air flow; b) spray apparatus; c) cap. impinger; d) total of 4 plates per experiment; e) No of bacteria; f) per 1 hr total operation time, g) per m^3 air; h) relative bacteria yield in %.

way it is possible to determine the bacteria content at a particular time in a continuous analysis of the air samples.

In the agar drum-centrifuge developed in our laboratory (see Fig. 1, VII) we tried to employ a combination of these two functional principles.

As should be obvious from the figure; a chamber is present in the inner side of the stationary housing into which an agar drum is placed; the chamber and drum are driver by an electric motor (2 000-2,500 rpm) which is situated on the outside of the housing. An axially located slit casing extends from the bottom of the chamber into the area

of the drum; this slit casing only rotates at the rate of one revolution per minute by means of the relative drive of a rotating delay mechanism. The air intake line which enters into the interior of the drum has been installed in such a manner that both of its nozzle arms are vertically movable in the slot casing. This air supply line is threaded on the outside and fits into a special socket in lid. As a result of the rotation of the air supply line which is retarded by a slit casing mounting, both of these nozzles gradually move towards the top by means of these threads. In this fashion the nozzle whose distance from the agar drum and nozzle opening is adjustable, moves along the agar drum wall in the space of about 15 minutes in a spiral fashion. The exhaust air line is installed in the upper housing cover lid onto which a Wright respirometer is mounted. The air flow occurs by means of an aspirator which is connected to the line and which can be regulated with a valve.

A prerequisite for the work with such an agar-drum-centrifuge was an instrument, which can be used to layer the drums with an agar medium under sterile conditions. This is possible with an agar pour-centrifuge which we ourselves have designed and built (see Fig. 1, VIII A).

The liquid nutrient agar (about 55°C) is poured into the angular agar filling line which reaches into the inner area of the drum. This agar-pour-instrument can also be used as a so-called horizontal centrifuge as well as for the collection in air bacteria (see page 35). The air intake line which reaches into the interior of the drum in a horizontal fashion when the instrument is used as a collection apparatus for air bacteria, is closed when the agar is being poured.

Our first determinations of the bacteria content of air with this vertical agar drum centrifuge were disappointing. As an example of this we can refer to Table 18. The results shown in this table were obtained with two comparative experiments involving two such instruments, one slit sampler and one Krotov apparatus. The bacteria yield obtained with these two last mentioned collecting instruments was three to four times higher than with our centrifuge; we should further note that with an optimal air flow of only five liters per minute (see Tables 1 & 7) far better values would have been obtained with the American and Russian instruments. Furthermore we have to consider that with the drum centrifuge a much larger agar area is available (535 cm^2 as compared to 152 cm^2 or 75cm^2) but, nevertheless, the bacteria yield in this apparatus was only 20 to 25% that of the slit sampler or the Krotov instrument.

Table 18

Comparative determinations on the air bacteria content by means of a vertical centrifuge (closed system, agar drum, relative drive of air jet), slit sampler and Krotov instrument

(Mean values of 2 comparative experiments)

Experimental area: large laboratory
experimental duration: 15 min each

	ⓐ Vertikal-Zentrifuge I	Vertikal-Zentrifuge II	Slit-Sampler	ⓑ Krotov-Gerät
ⓒ Düsen- bzw. Schlitzöffnung	2,5 mm	5,0 mm	0,2 mm	.
ⓓ Luftdurchfluß pro Min.	15 l	15 l	18 l	20 l
	ⓖ Luftkeimzahlen (Mittelwerte)			
ⓔ pro Agarfläche	38	38	149	215
ⓕ pro m^3 Luft	170	170	552	718

[Legend]: a) vertical centrifuge; b) Krotov instrument; c) jet or slit opening; d) air flow per min; e) per agar area; f) per m^3 air; g) mean values of the numbers of air bacteria.

Even with modified experimental conditions, for instance, in the air flow volume, nozzle width, nozzle to agar distance, no better results were obtained. When the air supply line was closed and only the agar drum was left to rotate, or when the two round nozzles were substituted by slot nozzles with a height of 8 cm (i.e. they covered the entire height of the drum) and a slit width of 1 mm, even worse results were obtained.

Similar unsatisfactory results were obtained when we substituted the agar drums by liquid drums (75 ml of NaCl solution per drum) by using slowly rotating slit nozzles; the corresponding drums have a liquid collection grill in

their lower margin. With the hope for better results we permitted the ambient air to enter into the drum area by removing both covers for the chamber and the centrifuge housing. This way we obtained a bacteria yield which was on the average double that obtained in similar simultaneous experiments with the slit sampler. We still have to perform comparative experiments with the impinger procedure.

The most impressive bacteria yield was obtained when a rotating slit nozzle and agar drum were used in the open vertical centrifuge. With an operation of only two minutes we obtained 2,000 to 5,000 bacteria colonies per drum, i.e. on the average, about five times the average yield of the agar plate of a slit sampler (see page 38).

VIII. Determination of Air Bacteria Content With A Horizontal Centrifuge Constructed In Our Laboratory

Since the vertical centrifuge with its rotating air supply by means of a relative drive did not fulfill our expectations, we undertook more detailed examinations of the simpler horizontal centrifuge. Thus, we partially modified the apparatus which was used for the agar-drum-instrument (see Fig. 1, VIII) or its functional basic design (see Fig. 1, VIII C).

In contrast to the vertical centrifuge, in which a chamber containing the drum rotates within an immobile centrifuge housing, the horizontal centrifuge only has one rotating housing chamber with functions as a drum container which can be closed by means of a screw cover. Since the electromotor only permitted the variation of very low revolutions, all experiments were carried out with a drum rotating at 1600 to 2400 rpm.

Our first experiments were carried out with the agar pour-instrument (see Fig. 1, VIII A).

For this purpose the agar filling line was closed. The air supply occurred via a pipe reaching axially into the drum area into the proximity of the air foils. The exhaust air left via eight narrow slit-like openings which were situated along the outer rim of the screw cover (similar to the apparatus shown Fig. 1, VIII B).

In comparison this instrument with the Krotov instrument we were first interested in finding out how the duration of the experiment (2, 4, 8 and 16 minutes) affects the bacterial yield. The average values obtained with five parallel

experiments are shown in Table 19 and show that with a horizontal centrifuge (a modified agar-drum-pour-instrument) the bacteria yield obtained was 3 to 5 times greater than that obtained with the Krotov instrument; in both cases no proportional relationship was found between experimental duration and bacterial yield. One can explain these lower bacteria counts by the fact that these experiments were carried out in the relatively bacteria-poor evening hours.

We have obtained an even better bacteria yield with this agar drum-pour-instrument when the slit cover was replaced by metal discs of different sizes, which were immobile and were installed in front of the drum housing; the diameter of these metal discs was about 7 to 14 mm smaller than the drum diameter (see Fig. 1, VIII B). With an air supply volume of 15 liter per minute, an operation time of two minutes and an optimal drum to disc surface to slit distance of five mm, we obtained bacteria counts of 1700 to 2600 per m^3 of air. Since in this modification the air not only entered through the air supply line but obviously also through the anullar drum/disc slit which actually served for elimination of the exhaust air, the exact measurement of the air sample volumes was only an illusion. For this reason we carried out no further experiments with this instrument.

In order to guarantee a more exact measurement of the air sample volumes we surrounded the rotating drum housing with an immobile cylinder containing the exhaust line which was connected to the cover chamber containing an air supply line in such a manner that there was a distance of one centimeter between the chamber and the drum through which the air could pass (see Fig. 1, III C). In comparative experiments with the Krotov instrument and using an experimental duration of ten minutes with a uniform air flow of 20 liters per minute, we found, however, that this modified horizontal centrifuge only yielded 80% of the bacteria count obtained with the Krotov instrument.

The air intake funnel shown in Fig. 1, VIII D proved to be a lot better than this cover chamber. Thus, it appeared in corresponding comparative experiments using this instrument and the slit sampler, that with an operation time of two minutes and an air flow of five liters per minute, on the average 2,000 bacteria per m^3 of air were obtained with the former instrument; this is twice the bacteria yield which could be obtained with the latter instrument. It does not seem impossible that the efficiency of the horizontal centrifuge can be increased by modifying the air foils and the exhaust shield mounted on the funnel.

Table 19

Comparative Determinations of Air Bacteria with an Agar-drum-pour-instrument (slit exit) Krotov Instrument with Various Experimental Durations

(Mean values of 5 comparative experiments each)

Experimental area: large laboratory
air flow: uniformly 12.5 l/min

	(a) Agartrommel-Gießgerät		(b) Krotov-Gerät	
(c) Laufzeit	(f) Keimzahlen (d) pro Trommel	(e) pro m^3 Luft	(g) pro Platte	(e) pro m^3 Luft
2 min.	7	280	2	80
4 "	11	220	2	40
8 "	14	140	5	50
16 "	31	155	7	35

[Legend]: a) Agar-drum-pour instrument; b) Krotov--Instrument; c) experimental duration; d) per drum; e) per m^3 air; f) No. of bacteria; g) per plate.

Since we obtained an above average bacteria yield with the open vertical centrifuge having a rotating slit nozzle (see page 36), we repeated these experiments and, at the same time, undertook parallel experiments with an open horizontal centrifuge (see Fig. 1, VIII E). This way we could find out at the same time whether there was a relationship between operational duration (1, 2, 4, 8, and 16 minutes) and bacteria yield. During the 50 minutes of the total duration of these comparative experiments a slit sampler having an air flow of 5 liters per minute was also in operation as a control, but since the same agar plate was used to collect the bacteria, the clock work had to be wound every 15 minutes. As one can see from the results given in Table 20, already after one minute of operation both drums of the vertical and horizontal centrifuge showed approximately 1100 or 480 bacteria colonies. Up to an operation time of 8 minutes (with bacteria count of about 7,000 ot 4,200 per drum) the

bacteria yields increased in both cases in proportion to the starting time.

Table 20

Comparative Determinations of Air Bacteria with an Open Vertical Centrifuge Having a Rotating Slit Nozzle, an Open Horizontal Centrifuge and Slit-Sampler at Various Experimental Durations

Experimental area: large laboratory
Revolutions in vertical centrifuge: 2.400 U/RPM
Revolutions in horizontal centrifuge 1.600 U/RPM
air flow: be in Slit-sampler: 5/1 min.

a) Versuchs-dauer	b) Vertikal-Zentrifuge	c) Horizontal-Zentrifuge	Slit-Sampler
	d) Keimzahlen/Agaroberfläche.		
1 min.	1100	480	•
2 "	2100	1050	•
4 "	3600	1700	•
8 "	7000	4200	•
16 "	8000	4200	•
50 min.	•	•	110

[Legend]: a) experimental duration; b) vertical centrifuge; c) horizontal centrifuge; d) No. of bacteria/agar surface.

This result is worthy of consideration since in none of the other bacteria collectors tested by us, such a good proportional relationship between operation time and bacteria yield was obtained. Thus in comparison only 110 bacteria were collected by the slit sampler in 50 minutes, which corresponds to a relative content of 440 bacteria per m^3 in the air. With the vertical centrifuge 10 or 16 times as many bacteria were collected in one or 8 minutes than with the slit

sampler over the period of 50 minutes. Figure 3 a, b, and c are photographs of the corresponding agar drums or the agar plate of the slit sampler. In spite of this superiority these open air centrifuges have, however, the disadvantage that no measurement of the air sample volume is possible. So far our experiments with slit covers, cover discs, cover chambers or air intake funnel for obtaining such measurements led to a more or less decreased bacteria yield. For this reason research is in progress to obviate these difficulties.

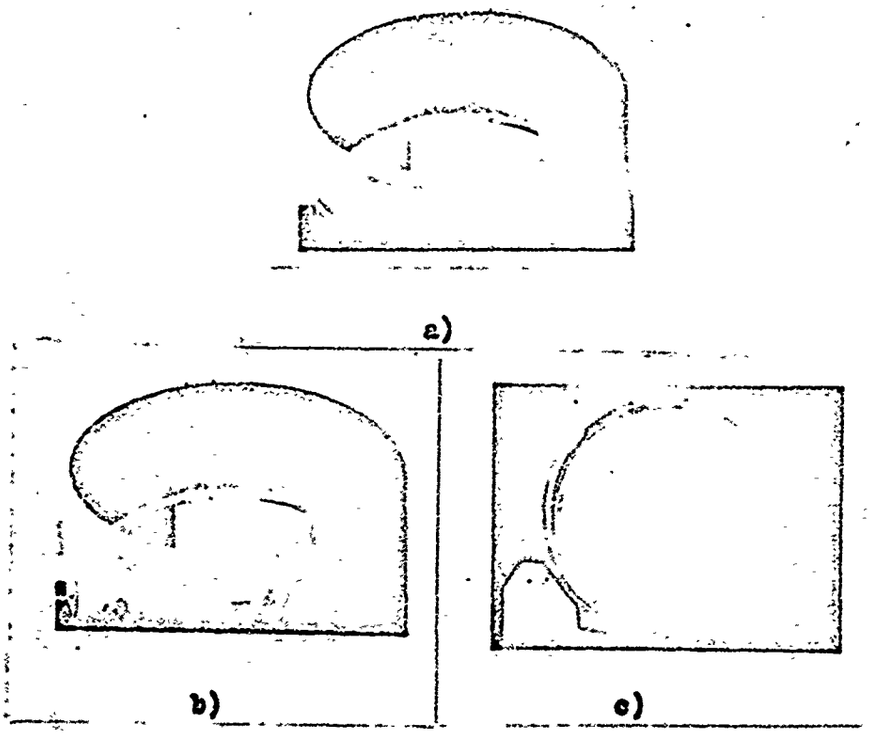


Fig. 3.

IX. Installation of Accessory Instruments in the Airplane and Preliminary Determinations of the Air Bacteria Content at Different Flight Altitudes.

In order to take air samples at different flight altitudes, an air intake pipe was installed in the air foil of an airplane; this air intake pipe is then connected to a PVC hose leading into the cabin. On the air foil we also

installed a thermometer and an instrument to measure humidity and whose remote dial was mounted in the cabin. As an instrument for measuring the humidity we used one produced by the Weiss Company in Giessen, which was very sensitive to fluctuations in humidity and reacted in one or two seconds in the vicinity of the ground, but had a much slower function in the airplane; for example, twenty to thirty seconds after having flown through a cloud this instrument registered the lower humidity outside the cloud.

Considering the relatively low bacteria yield which we obtained with the instruments tested in our laboratory, except our open air centrifuges, we could not expect at the start that we would find any especially impressive data at the higher flight altitudes. This was verified with a total of eight flights which occurred at altitudes of 1,000 to 7,000 feet and occurred, for the most part, over in the area of Marburg and its surroundings and also in the area south of Stuttgart and northwest into the Ruhr area. For these preliminary experiments we first used the slit sampler (Fort Detrick). Since we were interested in possibly getting a large bacteria count per agar plate, we generally chose a large air flow which necessarily led to relatively low and rather invalid values of bacteria per m^3 of air; (this occurred as a result of small conversion factors).

Table 21 shows the bacteria counts per plate or per experimental duration which were obtained in five different flights with a total of 36 determinations and a uniform air flow of about 40 liters per minute. In experiments 1 through 4a the obtained bacteria yield was very slight (0.3 to 5.2, bacteria per plate); quite a number of the agar plates showed no bacteria growth and with the presence of only a few bacteria colonies one cannot conclude with any degree of certainty whether these had or had not come from the air supply line. These sparse results were insufficient for any evaluation concerning flight altitude, air temperature and humidity, cloud conditions, wind direction, geographic and topographic conditions of the areas which were covered. We might give some consideration to test flight 4B which occurred through the very dusty air of the Ruhr area (vicinity of Castrop-Rauxel-Dortmund), where we obtained an average value of 31 bacteria per plate or operation time, which, on the other hand, agrees with all other known data in that, generally, air with a high dust content is relatively rich in bacteria. A modification in the width of the air supply slit (0.1 to 1 mm) while the air flow was also changed, at the same time, as was done in trial 5a and 5b, only resulted in a more marked scattering of the again very slight bacteria yields without showing any definite relationships.

Table 21

Determinations of Air Bacteria at Altitudes of 1,000-7,000 feet by means of a Slit-sampler (Fort Detrick)

ⓐ Versuchs-flug	ⓑ Anzahl der Bestimmungen	ⓒ Jeweiliger Luftdurch-fluß (ca.)	ⓓ Jeweilige Versuchs-dauer	ⓔ Durchschnittliche Keimzahl ⓕ pro Platte bzw. Versuchsdauer	ⓖ pro m^3 Luft
1.	5	40 l/min.	5 min.	0,8	(4)
2.	7	" "	" "	0,3	(1,3)
3.	7	" "	" "	5,0	(25)
4 a.	15	" "	10 "	5,2	(13)
b. *)	2	" "	" "	31,0	(77)
5 a. **)	6	13-65 l/min.	15 "	2 - 15	(4 - 29)
b. **)	6	6-35 l/min.	30 "	2 - 13	(4 - 15)

* Flight over the Ruhr area
** At varying slit widths (0-1 mm)

[Legend]: a) experimental flight; b) No. of determinations; c) air flow; d) experimental duration; e) average bacteria count; f) per plate or experimental duration; g) per m^3 of air.

In simultaneous comparative determinations at flight altitudes of 3,000 to 6,000 feet by means of Krotov instruments, slit samplers, and capillary impingers, the values shown in Table 22 were obtained; these data verify by far the other conclusions and previous comparative results (see Tables 11 and 17).

Corresponding experiments with the closed air centrifuges developed by us which allowed the measurement of the air sample volume, could not yet be performed since these instruments were available only a short period before our test flights this year and were needed for experimental purposes in the laboratory.

Discussion of the Results:

The more efficient an air bacteria collector is, the more bacteria are generally killed in the process, and if the collecting instrument is not very efficient no bacteria are caught. An exact statement on the absolute bacteria content in larger air volumes is, therefore, not possible with any instrument for in both cases an uncontrollable portion of the bacteria is never collected. Under very favorable conditions such values are only rough approximations of a 100% bacteria collection. For such statements comparative tests with approved bacteria collecting instruments and corresponding determinations of the bacteria content in the exhaust air are indispensible, but the results can finally only be applied to the present experimental conditions.

An efficient collecting instrument for air bacteria must have all of the following requirements: It must have a large capacity for collecting bacteria, which will act to the same extent in bacteria-rich or bacteria-poor air or close to the ground or in the higher altitudes of the atmosphere as well as at different humidities and air temperatures. With as high an efficiency as possible the different bacteria species, aerobes and anaerobes, vegetative bacteria or spores, as well as fungi, must be collected to the same extent. Especially in the case of bacteria free air the instrument must be able to process larger air sample volumes in a relatively shorter time, since only this way the large conversion factors needed for the calculation of the bacteria content per m^3 of air and the accompanying large uncertainty factors can be avoided. This is especially true for the determinations of the bacteria content with the airplane since under these conditions a reliable back calculation for the bacteria content of the air is possible over only a very narrow area. In addition a chance for collecting qualitatively

different bacteria increases with the size of the bacteria yield. Otherwise it is not possible to say with certainty whether these bacteria do or do not originate from the air supply when one is dealing with small numbers of bacteria.

In the literature numerous instruments for the collection of air bacteria are described which all work on different principles and can be modified in various ways (Bourdillon, R. B., Lidwell, O.M. and Thomas J. C., J. Hyg. (Cambr.) 41 (1941), 197; Lukiesh, M., Taylor, A.H. and Holladay, L.L. (1946), J. Bact. 52 (1946) 55; Proctor, B. W. and Porter, B.W., Science Press, Lancaster (1942), 48; Vlodavec, V.V., reference in Mitt. der Uebersetzergr. Ost - 300061; Wolf, H.W. et al. Public Health Mono. 60 (1959). The efficiency of the single apparatus is often evaluated in quite a different fashion and partly even in a complete opposite fashion. On the basis of a study of the literature, it is often impossible to arrive at one's own opinion since important factors are often missing in the publications or are only mentioned briefly, for instance, more detailed data concerning exact experimental conditions, adjustment of the instruments, determinations with the use of different air sample volumes, the number of single experiments which are the basis of the average values as compared with other instruments under the same conditions in simultaneous comparative experiments. Especially as far as most cases are concerned, there is no data concerning exhaust air controls. Very often the comparative values are based on experiments with aerosols containing bacteria or bacteria spores which, of course, would not permit any valid conclusion as far as corresponding results under normal conditions are concerned. It is possible that different instruments can have vastly different collection abilities with the different kinds of bacteria, or this would also presuppose the possibility for a maximal collection of certain bacteria with a certain instrument or, on occasions, a modification of the adjustments of the instrument (Bourdillon, R. B., Lidwell, D. M. and Thomas, J. C., J. Hyg. (Cambr.) 41 (1941), 197; Noller, E. and Spenellore, J.C., Appl. Microbiol. 4 (1956), 305: One should also compare the drastically different findings obtained in comparative experiments involving slit samplers and the Krotove apparatus with the use of aerosols containing Bac. subtilis; these findings were reported in a letter, dated March 25, 1964, which was written by H. N. Glassmann (Fort Detrick) for Dept. T III 7. Furthermore one has to consider in the work with bacterial aerosols that in the spraying of bacterial aerosol suspensions of known bacterial concentration, an uncontrollable portion of the bacteria would settle on the walls, on the floor and on the ceiling of the small closed room or they can settle anywhere over the course of the experiments. We

ourselves found that in our experiments in a small (250 l) closed incubator containing normal air during the simultaneous operation of two or more instruments, air-streams and turbulences originated which strongly influenced the normal efficiency of the single instrument in a different way. Furthermore one has to be careful that in comparative experiments in a large room the air taken in and the exhaust air of the single instruments do not influence each other and that there is sufficient distance between the instruments themselves as well as sufficient distance from the instruments to the wall. In addition we have to add that only one certain nutrient medium is used for the cultivation of the collected bacteria; this way we also have different chances for the cultivation with different kinds of bacteria. For instance, we had the experience with certain air sarcina that in the cultivation in nutrient broth an addition of normal calf serum, in a dilution of 1 to 300, acted as a growth inhibitor. Altogether one can say that even with numerous comparative experiments only average values of the bacterial content can be obtained, which, nevertheless, seem to simulate valid results. The valid judgment of the results obtained by different authors or an evaluation of the instruments used by them is, for this reason, not always possible.

The different instruments for the collection of air bacteria ~~which we have employed~~ have been tested in regard to their possible use for taking air samples with an airplane. In the opinion that the efficiency of an effective instrument differs only in degree, but not in principle, we carried out our basic and comparative determinations on the air bacterial content in the laboratory, whereby we kept in mind the previously mentioned basic principles and considerations.

Since the establishment of the laboratory and the training of the personnel required time and since obtaining the different instruments for the air collection took up great amounts of time, we were by necessity forced to limit our experiments to purely quantitative determinations and comparatives tests of aerobic bacteria which multiplied in two or three days on blood nutrient agar at 37°C. Thus we did not consider any of the slower growing bacteria, the fungi or the bacteria which multiply only in strongly anaerobic conditions or those which require special nutrient media. The comparative examinations were always performed simultaneously under the same conditions. In order to obtain as valid and as average comparative values as possible, we always performed a larger number of single determinations. Thus, we believe that the results obtained by us have a high degree of validity.

Table 22

Comparative Determinations of our Bacteria At Altitudes of 3,000-6,000 ft With a Krotov Instrument, Slit Sampler and Capillary Impinger

(Average values of 6 comparative experiments)

	Krotov-Gerät	Slit-Sampler	Kap.-Impinger
Luftdurchfluß	20 l/min.	5 l/min.	2 l/min.
Versuchsdauer	10 min.	10 min.	30 min.
	durchschnittliche Keimzahlen		
pro Platte bzw. Gerät	6,0	3,7	30 +)
pro m^3 Luft	(30)	(74)	(500)

* in 150 ml collecting fluid

[Legend]: a) air flow; b) experiment duration; c) per plate or instrument; d) per m^3 air; e) Krotov instrument; f) cap. impinger; g) average bacteria count.

The experiments performed by us with a total of eight different instruments and several modifications often showed significant deviations from the results obtained by other authors. Thus it was shown, for example, that the optimal air flow of 38 liters per minute (= 1 foot per minute) or 30 liters per minute, as recommended by the manufacturer for the slit sampler (Fort Detrick) and the Krotov instrument, does not prove to be valid since we always obtained double the bacteria per m^3 of air with air sample volumes of five liters per minute; in this case the latter instrument was slightly superior to the former. To be sure, the rotameter of the Krotov instrument permitted no measurement of smaller air volumes and its electromotor did not permit constant operation under these conditions. It is also important to state that by installing an air intake funnel, which is more or less unavoidable for the taking of air samples by means of an airplane, the bacteria yield of a slit sampler is decreased so that under these conditions both instruments have approximately the same efficiency. Furthermore, we could

Table 23

Comparison of the Efficiency (air bacteria collecting capacity) of 10 Tested Instruments for the Collection of Air Bacteria

	ⓐ s.Abb.1	ⓑ absolute Keimausbeute in % (ungefähr)	©.vgl. Tabelle (Seite)
1. Vertikal-Zentrifuge (offen)	VII	?	20
2. Horizontal-Zentrifuge (offen)	VIIIc	?	20
3. Fritte-Impinger	IV	13	13
4. Horizontal-Zentrifuge (Agartrommel-Gießgerät)	VIIIa	10	19 (S.)36
5. Kapillar-Impinger	III	10	12 11 13 17
6. Horizontal-Zentrifuge mit Trichter	VIIId	5	
7. Krotov mit Trichter	I	2,5	8 11 15 19 (S.)36
8. Slit-Sampler mit Trichter	II	2,5	8 11 15 17 20
9. Sprüh-Gerät	VI	2,5	17
10. Gelatine/Membran-Filter	V	1	15

[Legend]: a) see Fig. 1; b) absolute bacteria yield in % (approximate); c) cf. table on page; 1) vertical centrifuge (open); 2) horizontal centrifuge (open); 3) fitted glass impinger; 4) horizontal centrifuge (agar drum pour instrument; 5) capillary impinger; 6) horizontal centrifuge with funnel; 7) Krotov with

funnel; 8) slit-sampler with funne; 9) spray instrument; 10) gelatine/membrane filter.

not verify the absolute bacteria collecting capacity of 98% which was reported by Windisch for the Bronn capillary impinger. While the low bacteria counts obtained values of 5-10% on the basis of the corresponding exhaust air test. This value of approximately 10% was used as a reference value and made possible an approximate evaluation, derived from the average values of the comparative experiments, or, as a result, the more or less absolute bacteria counts obtained with these instruments under normal atmospheric conditions. Table 23 lists ten instruments, including the modifications, in the sequence of their percentage bacteria collecting ability (these values correspond to the experimental data in the text). According to this table the gelatin membrane filter instrument with which we obtain a bacteria yield of only 1%, is the worst, which is not too surprising because even the manufacturing firm Sartorius, Gottening, called attention to the fact that poor results are obtained when collecting vegetative bacteria with this particular instrument: Goetz, A., Amer. Ind. Hygiene 10 (1955), 16: Albrecht, J., Arch. f. Hyg. 142 (1957), 210. With a yield of 2.5% it seems that the spray apparatus developed by us as an experimental model is similar in efficiency to the American and Russian slit sampler, but the Russian one has the advantage, because it is significantly faster and can yield results in a simpler fashion. The same can be said for the horizontal centrifuge (agar drum-pour-instrument) which has been developed by us in comparison with our fritted glass impinger having an absolute bacteria yield of approximately 10 or 13%. The best and fastest bacteria collecting capacity was found in our vertical and horizontal centrifuges even if they did not permit any measurement of the air flow volume. The superior efficiency of these instruments seems to erase this disadvantage when determinations of the air bacteria content are performed close to the ground, since with the high quantitative yield there is a much larger chance of collecting qualitatively different kinds of bacteria. In addition to the high efficiency of these instruments we can also mention the fact that with these instruments a proportional relationship or their further modifications, if possible with the device which can measure the air flow, are suitable for the determination of air bacteria counts with an airplane.

SUMMARY:

Eight different instruments for the collection of air bacteria, together with their several modifications, some of

which already are well known and others having been developed in our own laboratory, were tested in the laboratory under laboratory air conditions in the view of the possibility of their use in the determinations of the air bacteria content with an airplane. The experiments were limited to the determination of bacteria which multiplied aerobically at 37°C on predetermined nutrient media. By a thorough examination of the optimal experimental conditions and numerous comparative experiments and determinations of the bacterial content in the exhaust air, it was possible to compare the efficiency of the instruments with each other as well as their advantages and disadvantages. In this manner we arrived at conclusions which sometimes differed significantly from those reached by other authors. With an approximate bacteria collecting ability of 1% the gelatin/membrane filter instrument of the Sartorius works in Gotteningen was the least suited. Somewhat better and on equal terms with the bacteria yield of 2.5%, were our own spray instrument, the American slit sampler (Fort Detrick) and the Russian Krotov instrument. The agar drum-air-centrifuges which were built in our own laboratory with an adjustable air flow had an efficiency of 5 to 10% and thus corresponded to the Bronn capillary impinger as far as their capacity for bacteria collection is concerned. A home-made, simple fritted glass impinger having an efficiency of 13% was slightly more efficient than the capillary impinger. An efficiency which was some 50 to 100 times better than both the American and the Russian slit sampler and which was much more superior to all the other instruments as far as speed of bacteria collection is concerned, was possible with the air centrifuges designed and constructed in our own laboratory which, however, did not permit any regulation or measurement of the air sample volumes. The experiences gathered in our own laboratory experiments were expanded by preliminary orientational determinations of the air bacteria contents in the higher layers of the atmosphere by means of an airplane.

- END -